IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	TYAGI et al.	Examiner:	Unknown
Serial No.:	10/801443	Group Art Unit:	Unknown
Filed:	March 15, 2004	Docket No.:	11336.0020US01
Title:	IMPROVED PROCESS FOR PREPARATION OF 7-[a-AMINO (4-HYDROXYPHENYL) ACETAMIDO]-3-SUBSTITUTED-3-CEPHEM-4-CARBOXYLIC ACID		

CERTIFICATE UNDER 37 CFR 1.8:
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, with sufficient postage, in an envelope addressed to: Commissioner for Patents, Mail Stop Missing Parts, P.O. Box 1450, Alexandria, VA 22313-1450 on June 18, 2004.

By: AEwald
Name: A Ewald

SUBMISSION OF PRIORITY DOCUMENT

Mail Stop Missing Parts

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants enclose herewith one certified copy of an Indian application, Serial No. 1031/MUM/2003, filed October 3, 2003, the right of priority of which is claimed under 35 U.S.C. § 119.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: June 18, 2004

By _____
Douglas P. Mueller
Reg. No. 30,300

DPM/ame

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Government Of India
Patent Office
Todi Estates, 3rd Floor,
Lower Parel (West)
Mumbai – 400 013

THE PATENTS ACT, 1970

IT IS HEREBY CERTIFIED THAT, the annex is a true copy of Application and Complete Specification filed on 3/10/2003 in respect of Patent Application No.1031/MUM/2003 of LUPIN LTD., 159, CST ROAD, KALINA, SANTACRUZ (EAST), MUMBAI- 400 098, STATE OF MAHARASHTRA, INDIA, AN INDIAN COMPANY.

This certificate is issued under the powers vested in me under Section 147 (1) of the Patents Act, 1970.

Dated this 11th day of June 2004.

(A.T. PATRE)
ASST. CONTROLLER OF PATENTS & DESIGNS

FORM 1

THE PATENTS ACT, 1970

(39 of 1970)

APPLICATION FOR GRANT OF A PATENT

[See sections 5 (2), 7, 54 and 135 ; rule 39]

1. We, (a) LUPIN LTD., (b) 159, CST Road, Kalina, Santacruz (East), Mumbai – 400 098, State of Maharashtra, India, (c) An Indian Company,

2. hereby declare –

   (a) that we are in possession of an invention titled

   PROCESS FOR PREPARATION OF 7-[α-AMINO (4-HYDROXYPHENYL) ACETAMIDO]-3-SUBSTITUTED-3-CEPHEM-4-CARBOXYLIC ACID

   (b) that the Complete Specification relating to this invention is filed with this application.

   (c) that there is no lawful ground of objection to the grant of a patent to us.

3. further declare that the inventors for the said invention are:

   (a) TYAGI, Om Dutt
   (b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
   (c) An Indian national

   (a) RANE, Dnyandev Ragho
   (b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
   (c) An Indian national

1031 4977

1031/MUM/2003

3/10/2003

Received Rs. 300/- in Cash
Cheque/M.O./P.O. on 3/10/03
Vide Entry No. 4977 in the Register of Valuables, Bombay.
Date 3/10/03
Cashier

(a) SRIVASTAVA, Tushar Kumar
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) An Indian national

(a) SIRSATH, Krishnarao Tukaram
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) An Indian national

4. We claim the priority from the application(s) filed in convention countries, particulars of which are as follows :

NONE

5. We state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which we are the applicant/patentee:

NOT APPLICABLE.

6. We state that the application is divided out of our application, the particulars of which are given below and pray that this application deemed to have been filed on _____, under section 16 of the Act

7. That we are the assignees of the true and first inventors.

8. That our address for service in India is as follows :

S. MAJUMDAR & CO., 5, Harish Mukherjee Road, Calcutta - 700 025, State of West Bengal, Phone : 0-33-24557484/24557485/24557486 ; Fax : 0-33-24557487/24557488.

9. We the true and first inventors for this invention declare that the applicant herein is our assignee.

(a) TYAGI, Om Dutt
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) A US citizen

TYAGI, Om Dutt

(a) RANE, Dnyandev Ragho
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) An Indian national

RANE, Dnyandev Ragho

(a) SRIVASTAVA, Tushar Kumar
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) An Indian national

SRIVASTAVA, Tushar Kumar

(a) SIRSATH, Krishnarao Tukaram
(b) Lupin Ltd. (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune – 411 042, Maharashtra, India
(c) An Indian national

SIRSATH, Krishnarao Tukaram

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

11. Followings are the attachment with the application :
    (a) Complete specification (in quadruplicate).
    (b) Statement and Undertaking on FORM –3 duplicate.
    (c) Request for Examination (Form-19)- duplicate.
    (d) Copy of General Power of Attorney in our favor.
    (e) Fee Rs. 6000/- being Cheque bearing No. 946046
    Dated 01.10.2003 on Standard Chartered Bank.

We request that a patent may be granted to us for the said invention.

Dated this 01st day of October 2003.

MEGHNA VAIDYA
OF S. MAJUMDAR & CO.
Applicants' Agent

To
The Controller of Patents
The Patent Office
At Mumbai

FORM - 2

THE PATENTS ACT, 1970
(39 of 1970)
COMPLETE SPECIFICATION
(See section 10; rule 13)

1. TITLE

PROCESS FOR PREPARATION OF 7-[α-AMINO (4-HYDROXYPHENYL) ACETAMIDO]-3-SUBSTITUTED-3-CEPHEM-4-CARBOXYLIC ACID

2. (a) **LUPIN LTD.** (b) 159, CST Road, Kalina, Santacruz (East), Mumbai – 400 098, State of Maharashtra, India, (c) An Indian Company.

The following specification particularly describes the nature of this invention and the manner in which it is to be performed.

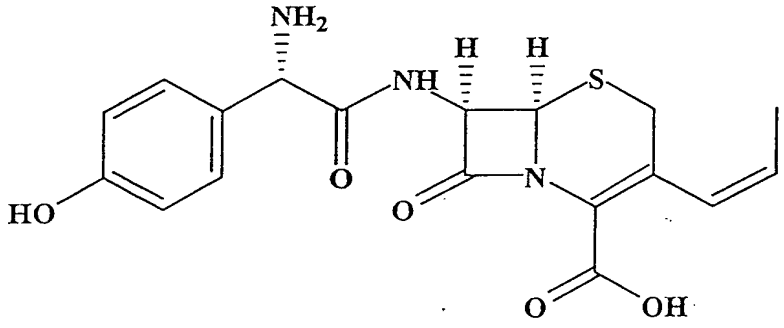
DUPL
1031/mum/2003

Complete specification treated as Provisional specification u/s 9(3) of the Patents Act, 1970

Jayant Anand.
(JAYANT ANAND)
Examiner of Patents & Designs.

Field of the Invention

The present invention provides a novel process for preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid viz. Cefprozil of formula (I) in high purity, substantially free of impurities.



(I)

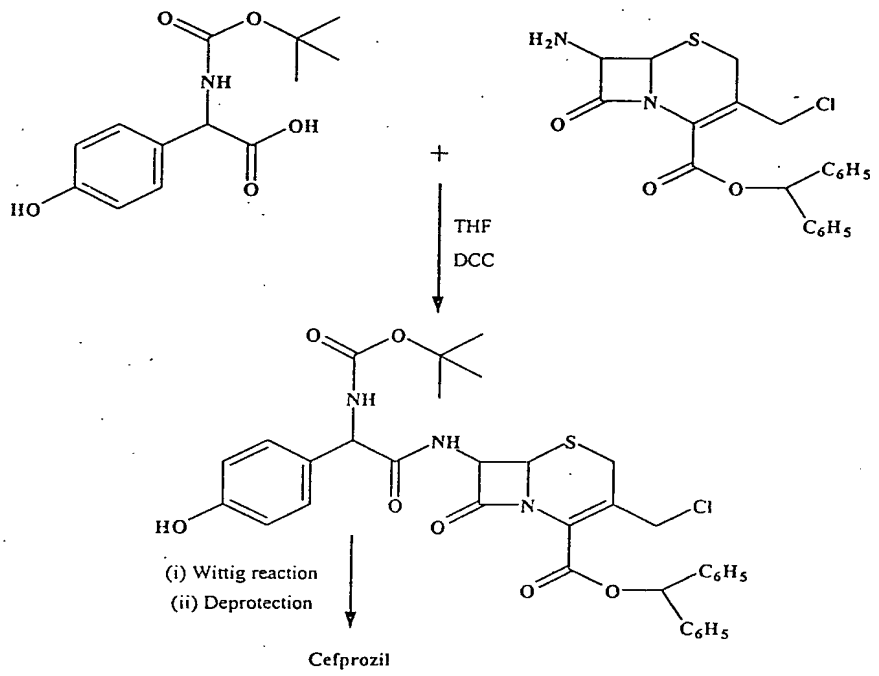
BACKGROUND OF THE INVENTION

Cefprozil, as disclosed in US 4,520,022, is a commercially valuable and therapeutically useful semi-synthetic, broad-spectrum oral cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram negative microorganisms.

Because of its therapeutic usefulness and broad, efficient spectrum of activity, there is always a need for an improved synthetic process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

In methods disclosed in prior art, synthesis of Cefprozil has essentially been carried out by amidification of a 7-amino-3-(1-propen-1-yl)-cephem derivative with α-amino-p-hydroxyphenylacetic acid or its reactive derivative as disclosed in the following patents.

U.S. 4,520,022 and U.S. 4,699,979 (Hoshi et al.) disclose a synthetic process for preparation of Cefprozil by condensation of benzhydryl-7-amino-3-halomethyl-3-cephem-4-carboxylate with D-2-(t-butoxycarbonylamino)-2-(p-hydroxyphenyl) acetic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) as the coupling agent and subsequent fuctionalization of the 3α position by Wittig reaction. The chemistry is summarized hereinbelow in Scheme I.



Scheme I: Synthesis of Cefprozil as per the method disclosed in U.S. 4,520,022 and U.S. 4,699,979

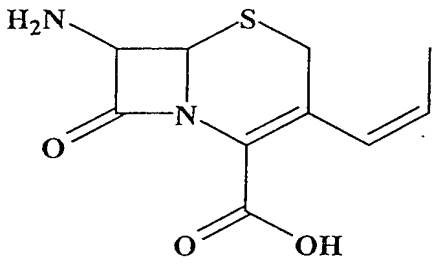
One of the limitations of the process is that it employs DCC which is toxic, expensive and requires rigorous anhydrous conditions. Also dicylohexylurea is formed as a byproduct during the process, removal of which calls for several tedious chromatographic purification and isolation steps to be employed to get the product in pure form.

U.S. 3,970,651, U.S. 3,985,747 and GB 1,532,682 disclose methods for preparation of Cefprozil, Cefadroxil and Cefatrizine which generally comprise reaction of 4-hydroxyphenylglycine with phosgene, followed by addition of gaseous hydrogen chloride to give 4-hydroxyphenylglycine chloride

hydrochloride. This is further reacted with a suitable 7-amino-3-substituted cephem derivative to give the desired cephalosporin antibiotic

However, these methods employ toxic and hazardous phosgene and gaseous HCl, which are difficult to handle on an industrial scale and cause environmental problem.

PCT application WO 98/04732 discloses a method for preparation of Cefprozil comprising reaction of 4-hydroxyphenylglycine with ethylene glycol to give an ester which is reacted with 7-Amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid (7-APCA), of formula (II), in presence of enzyme, acylase. However, this method utilizes excess amount of the expensive enzyme rendering the method uneconomical.



(II)

A salt of 7-APCA with amidine and its use in the production of Cefprozil is disclosed by Greil et al. in the PCT application WO03/011871. The application describes the production of Cefprozil by the reaction of an amidine salt of 7-APCA with a mixed carboxylic acid anhydride of a N-substituted-α–amino-p-hydroxyphenylacetic acid. The patent does not comment on the purity or yield of the product.

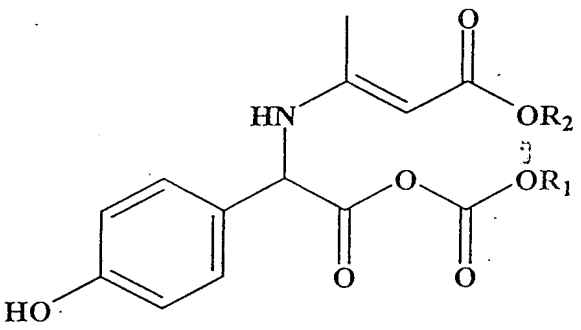
All the prior art methods discussed hereinabove are associated with the formation of varying amounts of impurities which affect the overall yield and the quality of the product. Also removal of these impurities calls for additional purification and isolation steps which render the process lengthy and tedious.

Regulatory authorities all over the world are becoming very stringent about the purity of an approved drug. Especially there is growing concern about the

nature and level of impurities present in such molecules. US Pharmacopoeia specifies that the purity of Cefprozil should be between 90 to 105 %. However, most of the prior art methods are associated with the formation of varying amounts of impurities and hence do not give product conforming to this criterion.

Cephalosporin antibiotics carrying the D-α-amino-α-(4-hydroxyphenyl) acetamido addendum at the 7-position such as Cefprozil and Cefadroxil are generally prepared by reacting the respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/derivative with an activated derivative of 4-hydroxyphenylglycine such as a reactive ester, a reactive amide or a mixed acid anhydride. However, use of reactive amide or esters makes it difficult to obtain the desired product in high purity and yield because of the occurrence of side-reactions as well as racemization.

Of the activated derivatives of 4-hydroxyphenylglycine, the mixed anhydride of α-amino-p-hydroxyphenylacetic acid of formula (III)



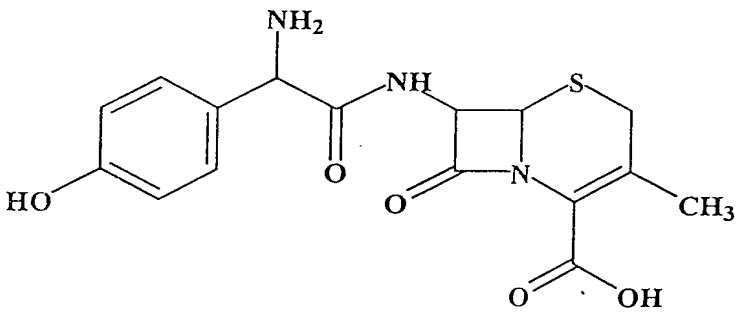
(III)

wherein R_1 is an alkyl or an aryl group and R_2 is methyl or ethyl,

is generally prepared by reacting N-substituted-α-amino-p-hydroxyphenylacetic acid or its salt (Dane salt) with an appropriate acylating agent at an appropriate temperature. For example in the process disclosed in US 3,985,741, the mixed anhydride is prepared by adding the acylating agent, base and the Dane salt to dry acetone at –10°C and stirring the slurry for 20 minutes. As per the process disclosed in US 4,218,474, the mixed anhydride is prepared by adding a chloroformate, such as ethylchloroformate, to a solution of N-protected-4-

hydroxy phenylglycine dissolved in an inert organic solvent at a temperature of –5° to 0°C in the presence of a base. According to the method disclosed in WO03/011871, the mixed acid anhydride is prepared by adding a base and Dane salt to an inert organic solvent at ambient temperature, cooling the suspension to –30°C, followed by addition of the acylating agent and stirring.

Most of the prior art methods for preparation of the mixed acid anhydride are associated with the formation of varying amounts of impurities. For example, during the course of the present invention, the inventors have reproduced the process for preparation of Cefprozil as disclosed in WO03/011871. It was observed that the preparation of mixed acid anhydride by the method reported in WO03/011871 and its subsequent reaction with amidine salt of 7-APCA is associated with the formation of impurities in the range of 6-7%. Preparation of mixed anhydride as disclosed in US 3,985,741 and its subsequent reaction with 7-aminodesacetoxycephalosporanic acid (7-ADCA) or a salt thereof gives 37% conversion to Cefadroxil of formula (IV) with impurities to the tune of 30-35 %.

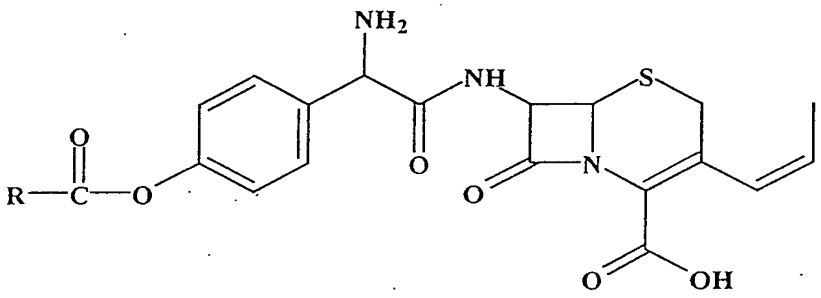


(IV)

In the prior art methods, cephalosporin antibiotics such as Cefprozil and Cefadroxil are prepared by reacting the mixed acid anhydride with respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/derivative such as an amidine salt of 7-APCA as disclosed in WO03/011871 and 7-ADCA or its salt as disclosed in US 3,985,741. However, use of 7-amino-3-substituted-3-cephem-4-carboxylic acid , its acid salt or an amidine salt as disclosed in these prior art methods is found to give the product in low yield due to side reactions of the 4-carboxylic acid group. Hence there is a need for a protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the

carboxylic acid group, which will not require additional deprotection steps and can be deprotected in-situ during reaction work-up.

One of the impurities observed during the preparation of Cefprozil by a method comprising preparation of a mixed carboxylic acid anhydride by acylation of Dane salt and its subsequent reaction with 7-APCA or a derivative thereof, is formed as a result of the reaction of the acylating agent with the 4-hydroxy group of the Dane salt. The impurity has the formula (V),



(V)

wherein R designates an alkyl, alkoxy or an aryl function derived from the acylating agent.

In summary, the prior art methods for preparation of Cefprozil:

i) utilize toxic and expensive chemicals such as phosgene, DCC and HCl,
ii) utilize expensive enzyme like acylase and
iii) are associated with formation of varying amounts of impurities which give the product in low purity and yield, rendering such methods less cost effective.

Therefore, a need exists for a simple and cost-effective method for the preparation of Cefprozil in high purity and yield. Such a need could be met through minimization of the impurities associated with the prior art methods with concurrent improvement in the purity and yield of the product.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide an improved method for the synthesis of Cefprozil of formula (I), which dispenses with the deficiencies of the prior art methods.

It is another object of the present invention to provide an improved method for preparation of Cefprozil in high purity and yield, substantially free of impurities.

It is yet another object of the present invention to synthesize Cefprozil in high purity, substantially free of impurities by a simple and cost-effective method which comprises preparation of mixed acid anhydride and its condensation with a protected 7-APCA.

It is also an object of the present invention to provide an improved method of preparation of mixed acid anhydride by selecting the sequence and temperature of addition of the reagents, which will result in minimization of impurities.

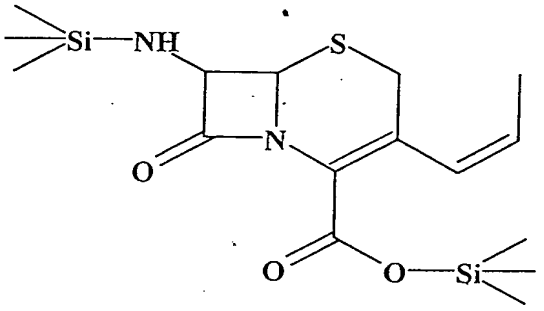
It is a further object of the invention to demonstrate the use of a silylated derivative of 7-APCA which further aids the minimization of impurities in the product and results in product of high purity.

SUMMARY OF THE INVENTION

One aspect of the invention relates to a simple, efficient, cost-effective method for manufacture of Cefprozil of formula (I) in high purity and yield.

Another aspect of the invention relates to a process for preparation of mixed acid anhydride of a N-substituted-α–amino-p-hydroxyphenylacetic acid, by selecting the sequence and temperature of addition of the reagents.

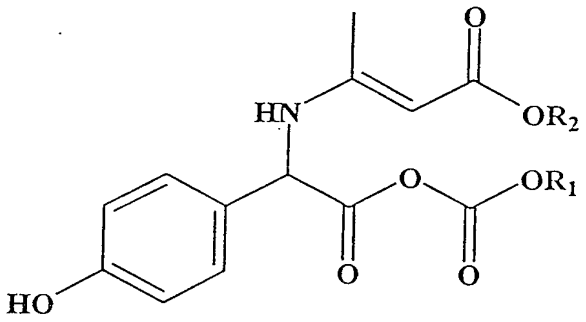
Another aspect of the invention is the use of disilylated 7-APCA of formula (VI) for the synthesis of Cefprozil.



(VI)

The objects of invention are best achieved by the process for preparation of Cefprozil in the form of a monohydrate in high purity in one aspect of the invention comprising steps of :

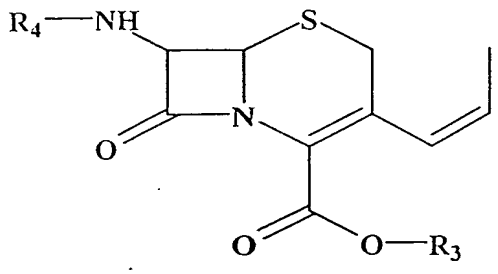
reacting a mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III)



(III)

wherein R_1 is an alkyl or an aryl group and R_2 is methyl or ethyl,

with a protected 7-amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid (7-APCA) of formula (VII)

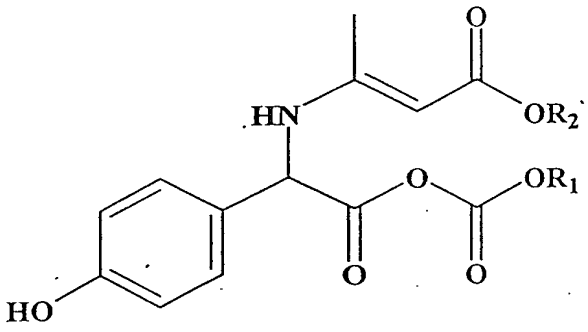


(VII)

wherein R_3 and R_4 are protective groups,
followed by hydrolysis, isolation and purification to give Cefprozil of formula (I) in the form of a monohydrate in high yield and purity, substantially free of impurities.

The mixed carboxylic acid anhydride of a N-substituted-α-amino-p-hydroxy phenylacetic acid or its salt (Dane salt) is prepared by selecting the temperature and sequence of addition of the reagents.

Thus according to a preferred aspect of the invention, there is provided a process for preparation of Cefprozil in the form of a monohydrate, comprising steps of:
reacting a mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III)



(III)

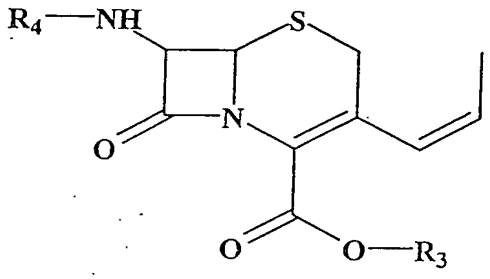
wherein R_1 is an alkyl or an aryl group and R_2 is methyl or ethyl, the mixed acid anhydride prepared by a process comprising the steps of

(a) adding an acylating agent and a base to an inert organic solvent or a mixture thereof at a temperature in the range of 0° to 40°C;

(b) cooling the solution to a temperature in the range of –70° to –30°C;

(c) addition of Dane salt of α-amino-p-hydroxy phenylacetic acid to the cooled solution and agitation at a temperature in the range of –70° to –30°C;

with a protected 7-amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid (7-APCA) of formula (VII)



(VII)

wherein R_3 and R_4 are protective groups,

followed by hydrolysis, isolation and purification to give Cefprozil of formula (I) in the form of a monohydrate in high yield and purity, substantially free of impurities.

Preferably the protected form of 7-APCA is the silylated form of 7-APCA. 7-APCA of formula (II) can be protected by silylation using a suitable silylating agent in an inert organic solvent or a mixture thereof over a wide temperature range, e.g. ambient to reflux temperature of the solvent system, preferably at the reflux temperature.

The mixed acid anhydride is condensed with the silylated compound at a temperature in the range of –90° to –30°C but preferably between –50° to –40°C and the silylated product is subsequently hydrolyzed to give Cefprozil in high purity, substantially free of impurities.

Cefprozil so obtained can be isolated preferably in the form of a solvate e.g. N,N-dimethylformamide solvate and subsequently purified to obtain Cefprozil in the form of a hydrate e.g. a monohydrate.

DETAILED DESCRIPTION OF THE INVENTION

The method for preparation of Cefprozil monohydrate in high purity, substantially free of impurities, as per the current invention comprises of the following steps;

A. Preparation of mixed carboxylic acid anhydride

The mixed acid anhydride, employed in the present invention has been prepared by an improved process which comprises of:

(a) adding an acylating agent and a base to an inert organic solvent or a mixture thereof at a temperature in the range of 0° to 40°C, preferably 20° to 25°C;

(b) cooling the solution to a temperature in the range of –70° to –30°C, preferably –35°C to –50°C;

(c) addition of Dane salt of an α-amino-p-hydroxy phenylacetic acid to the cooled solution and agitation at a temperature in the range of –70° to –30°C, preferably –35°C to –50°C.

In a specific embodiment of the present invention, the mixed anhydride is prepared from the N-substituted-α-amino-p-hydroxyphenyl acetic acid or its salt e.g. Dane salt such as sodium or potassium D-N- (1-methoxycarbonylpropene-2-yl)-α-amino-p-hydroxyphenyl acetate or sodium or potassium D-N-(1-ethoxycarbonylpropene-2-yl)-α-amino-p-hydroxyphenyl acetate. The Dane salt is activated with acylating agents such as reactive forms of aliphatic alicyclic or aromatic acids e.g. acid halogenides such as pivaloyl chloride and benzoyl chloride, and esters such including chloroformic acid alkyl esters, such as ethyl chloroformate. The activation is carried out in an inert organic solvent or a

mixture thereof, at a temperature in the range of 0° to 40°C, preferably 20° to 25°C followed by addition of a base and acylating agent to the solvent mixture at that temperature. The acylating agent is used in molar to slight excess with respect to the Dane salt, preferably 1-1.5 moles of acylating agent per mole of Dane salt are employed. The base is used in the range of 0.02-0.04 moles with respect to the Dane salt. The resulting mass is cooled to –70° to –40°C, preferably –35° to 50°C and the Dane salt is then added and the reaction is continued at this temperature for 60-180 minutes preferably 120 minutes. The final reaction mass containing the mixed anhydride is cooled to –75° to –60°C and maintained at this temperature for further reaction.

Examples of suitable acylating agents for the above process include reactive forms of aliphatic, alicyclic, or aromatic acids such as chloroformic acid, benzoic acid, pivalic acid and 2-ethylhexanoic acid. The reactive forms of these acids include their esters such as ethyl chloroformate, isobutyl chloroformate and their halogenides like pivaloyl chloride, 2-ethyl-hexanoyl chloride and benzoyl chloride. The preferred acylating agent is ethyl chloroformate.

In step A, a suitable base is employed as a catalyst selected from triethylamine, picoline, N-methylmorpholine, N,N-dimethylbenzylamine, lutidine, N,N-dimethyl-4-aminopyridine, N,N-dicyclohexylamine. The preferred base is N-methylmorpholine.

Suitable inert organic solvents or solvent mixtures employed for the reaction include, but are not limited to methylene chloride, tetrahydrofuran, chloroform, N,N-dimethyl formamide, diethyl ether, chlorotethane, acetonitrile, trichloroethylene, acetone and ethyl acetate. Preferably a mixture of a chlorinated solvent such as methylene chloride and N,N-dimethyl formamide is used.

B. Silylation of the 3-substituted-7-aminocephalosporanic acids

Use of silylated 7-APCA also forms another novel aspect of the present invention which further aids the minimization of impurities in the product and results in product of high purity.

Silylation of 7-APCA is preferably conducted in a suitable inert organic solvent or a mixture thereof, such as those used for the mixed anhydride preparation, by using known silylating agents over a wide temperature range, e.g. ambient to reflux temperature of the solvent system, preferably at the reflux temperature. The silylating agent may be used in molar equivalent or excess with respect to 7-APCA, preferably in a molar ratio of 1-2 with respect to 7-APCA. The reaction time is between 2-5 hours, preferably 4 hours. The silylated mass is cooled to –70° to –50°C prior to condensation with the mixed acid anhydride.

Silylating agents useful in the above process are known in the art [see, for example, U.S. Pat. Nos. 3,654,266, 3,575,970, 3,499,909, 3,595,855, 3,249,622 and U.K. Pat. Nos. 1,339,605, 959,853 and 1,008,468]. Any appropriate silylating agent known in the art could be employed. Examples of suitable silylating agents include hexamethyldisilazane, hexaethyldisilazane, trimethylchlorosilane, triethylchlorosilane, methyltrichlorosilane, methyldiethylchlorosilane, dimethylethylchlorosilane, triethylbromosilane, tri-n-propylchlorosilane, bromomethyldimethylchlorosilane, tri-n-butylchlorosilane, triphenylfluorosilane, hexa-p-tolyldisilazane, triphenylsilylamine, phenylethylmethylchlorosilane, phenyldimethylbromosilane, hexaphenyldisilazane, N-ethyltriethylsilylamine, tetraethyldimethyldisilazane, N,O-bis-trimethylsilyl acetamide, tetramethyldiethyldisilazane, or mixtures thereof. The most preferred silylating agents are N,O-bis-trimethylsilyl acetamide, trimethylchlorosilane and hexamethyldisilazane or a mixture thereof.

C. Condensation

The silylated mass prepared as in step B is added to the mixed anhydride mass prepared as in step A at a temperature in the range of –90° to –30°C but preferably between –70° to –60°C. After the addition is completed, the reaction

mass is agitated at –60° to –30°C, preferably –50° to –40°C till quantitative conversion to the silylated Cefprozil is achieved. The reaction time required for the reaction to go to completion could be between 2 to 5 hours, preferably 4 hours.

The level of impurities in the reaction mass is monitored at this stage. It is observed that the amount of carbonate impurity of structure (V) wherein R is ethoxy group as well as other impurities vary with the sequence of addition and temperature of addition of the reagents during mixed anhydride preparation. Also in the prior art methods, the mixed acid anhydride has been reacted with 7-APCA or a carboxylic salt thereof or with an amidine salt of 7-APCA. The selection of silylated derivative of 7-APCA over the other salts as well as the selection of temperature and sequence of addition of reagents for mixed anhydride preparation is established through comparison of experimental results as stated below.

For example, If the mixed anhydride is prepared by the method as described in WO03/011871, which comprises of addition of 4-picoline and Dane salt to a mixture of DCM and DMF, cooling the suspension to –30°C followed by addition of the acylating agent and agitating the suspension at –25° to –20°C and cooling it to –50°C, followed by its reaction with an amidine salt of 7-APCA, then 6-7% impurity is observed in the reaction mass.

The sequence of addition of reagents during mixed anhydride preparation influences the amount of impurity formed. If in the present invention, the sequence of addition of the reagents during mixed anhydride preparation is altered in such a way that the Dane salt is first added to a solvent or a mixture thereof at –50 °C, the temperature of the suspension is raised to 20° to 25°C followed by addition of acylating agent and then base, then the total impurities amount to 4.6% as against the 2.26% impurities observed if the sequence of addition is as described in the Step A of the present invention. If the Dane salt is first added to a solvent or a mixture thereof at –50 °C, the temperature of the suspension is raised to 20° to 25°C followed by addition of base and then the acylating agent, then the total impurities observed amount to 2.94%. This

illustrates that the sequence of addition of reagents in the mixed anhydride preparation affects the formation of impurities in the reaction.

The temperature of addition of reagents during mixed anhydride preparation is equally important in suppressing the amount of total impurities formed. For example, in the present invention, if the addition of the reagents in mixed anhydride preparation is done sequentially as described in step A , but at a uniform temperature of 20° to 25°C for all the steps (a), (b) and (c) followed by its reaction with a silylated derivative of 7-APCA, then the impurities formed add up to 18 % and conversion to the product is reduced to 4%.

If step (a) of mixed anhydride preparation is conducted at 50°C, the suspension cooled to –35° to –50°C followed by addition of the Dane salt and agitation at –35° to –50°C followed by its reaction with a silylated derivative of 7-APCA, then the total impurities to the tune of 7.73 % are observed in the reaction mass.

If all the steps (a), (b) and (c) of mixed anhydride preparation are conducted at –50°C, then the impurities are reduced to 3.68 %.

The total impurities are reduced to 2.26 % when in the mixed anhydride preparation, addition of an acylating agent and a base to an inert organic solvent or a mixture thereof is conducted at 20° to 25°C, the suspension cooled to –35° to –50°C followed by addition of Dane salt and agitation at –35 ° to –50°C and the mixed anhydride thus prepared is reacted with a silylated derivative of 7-APCA. Also at this selection of temperature and sequence of addition of the reagents, the formation of carbonate impurity of structure (V) wherein R is ethoxy is reduced to 0.2 % as against 1-1.5 % for other temperature combinations.

The qualitative results as monitored after condensation reaction by HPLC are tabulated hereinbelow.

Comparison of the method of present invention with the method as disclosed in WO 03/011871 and reproduced by the inventors

HPLC monitoring method results				
Process	Unconverted starting material %	Product %	Total impurity in reaction mass, %	Yield of isolated Cefprozil monohydrate, %
Present invention	4.99	92.74	2.26	70.7
WO 03/011871	10.23	76.29	6.4	62.8

Effect of sequence of addition of reagents in preparation of mixed anhydride on the level of impurities

HPLC monitoring method results				
Sequence of addition of reagents during mixed anhydride preparation.	Unconverted starting material %	Product %	Carbonate impurity (V) formed during reaction %	Total impurity formed during reaction, %
As per present invention	4.99	92.74	0.3	2.26
Dane salt added to solvents first followed by base and acylating agent	2.97	94.09	1.81	2.94

Dane salt added to solvents first followed by acylating agent and base	5.10	90.3	2.31	4.6

Effect of temperature in preparation of mixed anhydride on the level of impurities

Reaction temperature		HPLC monitoring method results			
Step (a) temperature*, °C	Step (c) temperature #, °C	Unconverted starting material %	Product %	Carbonate impurity (V) formed during reaction %	Total impurity formed during reaction, %
20-25	20-25	77.59	4.42	1.43	18
45-50	-35 to -50	3.56	88.71	1.41	7.73
-50	-35 to -50	1.07	95.25	1.03	3.68
20-25	-35 to -50	4.99	92.74	0.3	2.26

* temperature of addition of acylating agent and a base to an inert organic solvent or a mixture thereof.

temperature of addition of Dane salt of α-amino-p-hydroxy phenylacetic acid to and agitation of the reaction mass.

The HPLC method details are as under:

Mobile phase:

(A) Buffer and Acetonitrile (90:10).

(B) Buffer and Acetonitrile (30:70).

Both adjusted to pH 4.4 by orthophosphoric acid

Chromatographic system :

Column : Kromasil C-18 (4.6 X 250 mm), 5µ

Flow rate : 1.0 ml/minute. Auto sampler temperature: 10°C

Detector : UV at 280 nm. Injection volume : 20 µl.

Run time : 90 minutes. Temperature : Ambient.

Time program :

Time(min)	Mobile phase (A)	Mobile phase (B)
0.01	100	0
10.0	100	0
40.0	0	100
70.0	0	100
80.0	100	0
90.0	STOP	

The above experiments indicate that the temperature of addition and the sequence of addition of the reagents during mixed anhydride preparation influences the amount of impurity formed as also the product yield. The best selection of temperature and sequence of addition of reagents for the mixed anhydride preparation is addition of acylating agent and base to the solvent/s at a temperature of 20° to 25°C, cooling the solution to -35 ° to -50°C, followed by addition of the Dane salt and conducting the reaction at -35 ° to -50°C. Also, use of disilylated 7-APCA is a selection as it results in lesser impurities than those associated with the use of other derivatives of 7-APCA such as an amidine salt.

The cephalosporanic acid obtained in the protected form may be deprotected by appropriate methods e.g. by adding dilute mineral acid such as hydrochloric acid, sulphuric acid, nitric acid, but preferably dilute hydrochloric acid. The aqueous layer may then be diluted with a solvent and optionally the solid impurities may be removed at this stage of the process by treatment with activated carbon and/or filter aid.

D. Isolation and Purification

The product obtained in step C is isolated and purified by conventional methods. Particularly, the desired Cefprozil monohydrate may be obtained in its pure and crystalline form through its solvate preferably in the form of an N,N-

dimethylformamide solvate. The DMF solvate is prepared by the process as disclosed in US 4,694,079, which comprises of adding chilled N,N-dimethylformamide to the aqueous layer over a period of 30 minutes while maintaining the temperature between 10° to 15°C. The pH of the filtrate is adjusted to 4-7, by slow addition of an inorganic base selected from aqueous ammonia, sodium bicarbonate, sodium hydroxide but preferably aqueous ammonia solution. The solution may then be optionally seeded with the solvate crystals. The solution is agitated at 25° to 30°C to achieve complete crystallization of the DMF solvate. The resulting solid is filtered and washed with solvent preferably DMF and/ or ethyl acetate to obtain the DMF solvate of Cefprozil.

Desolvation may be carried out by dissolving the DMF solvate of Cefprozil in water or a mixture of water and an organic solvent such as acetonitrile, ethyl acetate, acetone or a C_1 to C_5 alkanol at a temperature of 0° to 20°C. The solution may be optionally seeded with the crystalline product and the slurry is agitated for 30-90 minutes. The solid product so obtained is filtered and may be washed with water and/or a solvent. The solid product is finally dried under vacuum at 30° to 45°C to obtain high purity Cefprozil of formula (I) in the form of a hydrate e.g. a monohydrate in high purity, conforming to pharmacopoeial specifications.

The improved process resulted in a significant improvement in the yield and quality of the product. The total impurities could be reduced from 6-7%, in the reaction mixture, associated with the prior art method to 2.26 % and from 0.8%, in the final isolated product to 0.48 %. The process results in a significant improvement in the yield of Cefprozil monohydrate from 62.8 % in the prior art method to 70.7 % in the improved process.

The above process could be extended to the synthesis of Cefadroxil in high purity and yield by condensation of the mixed anhydride of α-amino-p-hydroxy phenylacetic acid with disilylated 7-aminodesacetoxycephalosporanic acid (7-ADCA).

The following examples are given by way of illustration of the present invention.

Example-1a

7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyldisilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silyated 7 –amino-3-(propan-1-yl)-3-cepham—4-carboxylic acid (7-APCA)compound

Example –1b

To a mixture of methylene chloride (125 ml) and N,N-dimethylformamide (85 ml) and cooled to 20-25°C, is added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (12.72 g, 0.117 mole in 10 ml) under stirring. The resulting solution is cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (33.14 g, 0.11 mol) is added to it. The suspension is agitated at –40° to –35°C for 120 minutes. The reaction mass which is a solution of mixed anhydride product is cooled to –70 °C for condensation.

Example –1c

To a solution of the mixed anhydride product of procedure 1b, cooled to –70 °C, is added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 1a. The reaction mixture is stirred at –50° to –40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil is achieved. The reaction time is about 4 hours. The resulting reaction mass is added to a mixture

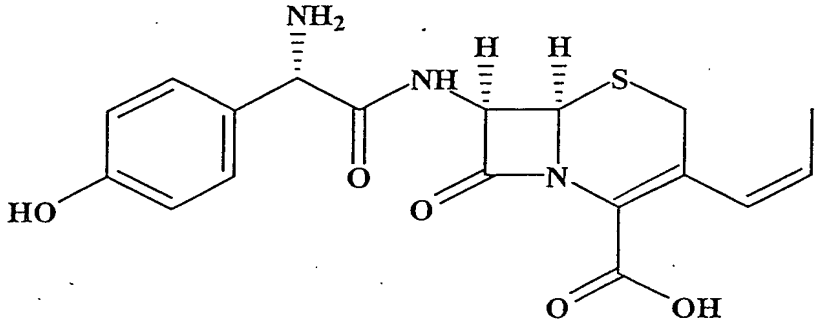
of 85 ml water and 20 ml concentrated hydrochloric acid maintained at –40° to –20°C. The temperature of the reaction mass is raised to 5° to10°C and the pH of the solution is adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass is stirred for 30 minutes and the layers are separated. The aqueous layer is diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature is maintained at 10° to15°C.

The aqueous solution containing Cefprozil as obtained above is converted to its DMF solvate as per the method disclosed in US 4,694,079. The wet DMF solvate without drying is desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product is filtered and dried to give Cefprozil monohydrate in the form of an off white to pale yellow crystalline powder.

Yield: 30 gms, % Yield: 70.7, Purity: 101.2%, Total impurities: 0.45%

WE CLAIM

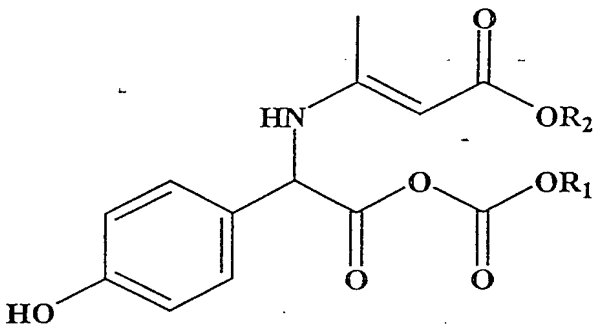
1. In a process for preparation of Cefprozil of formula (I)



(I)

in the form of a monohydrate comprising of

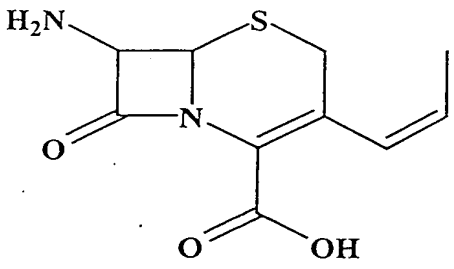
reacting a mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III)



(III)

wherein R_1 is an alkyl or an aryl group and R_2 is methyl or ethyl,

with 7-amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid of formula (II) in the form of a salt or derivative thereof,



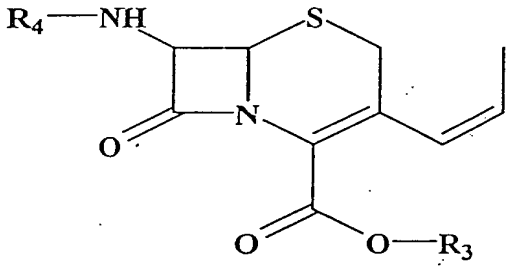
(II)

followed by isolation and purification of the product, the improvement comprises of

i) preparing the mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III) by a process comprising the steps of

(a) adding an acylating agent and a base to an inert organic solvent or a mixture thereof at a temperature in the range of 0° to 40°C;
(b) cooling the solution to a temperature in the range of –70° to –30°C;
(c) addition of Dane salt of α-amino-p-hydroxy phenylacetic acid to the cooled solution and agitation at a temperature in the range of –70° to –30°C;

ii) reacting the mixed acid anhydride of formula (III) thus prepared with a protected 7-APCA of formula (VII)

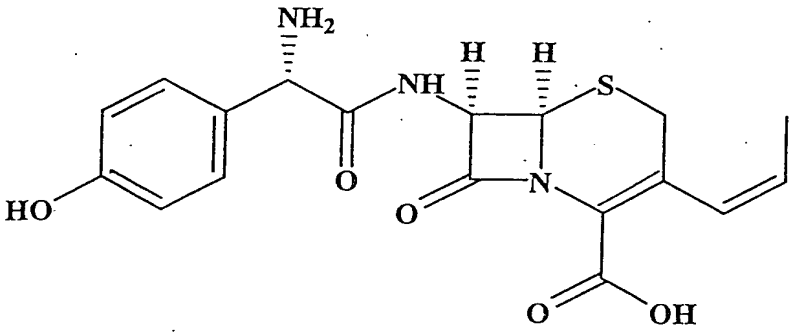


(VII)

wherein R_3 and R_4 are protective groups,

followed by hydrolysis, isolation and purification to give Cefprozil of formula (I) in the form of a monohydrate in high yield and purity, substantially free of impurities.

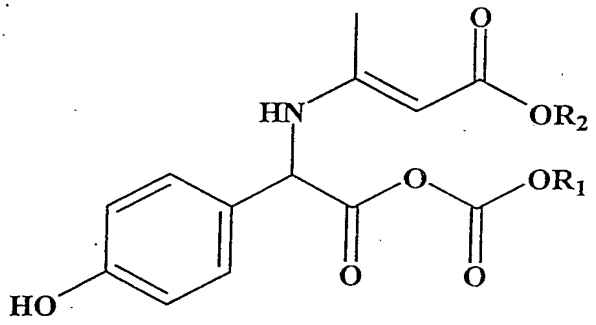
2. A process for preparation of Cefprozil of formula (I)



(I)

in the form of a monohydrate, according to claim 1, comprising of

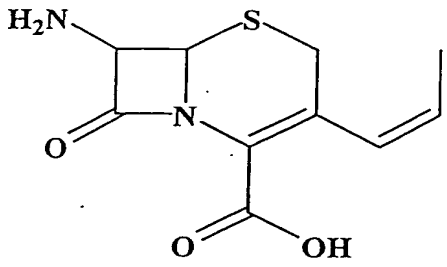
reacting a mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III)



(III)

wherein R_1 is an alkyl or an aryl group and R_2 is methyl or ethyl,

with 7-amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid of formula (II) in the form of a salt or derivative thereof,



(II)

followed by isolation and purification of the product, the improvement comprises of

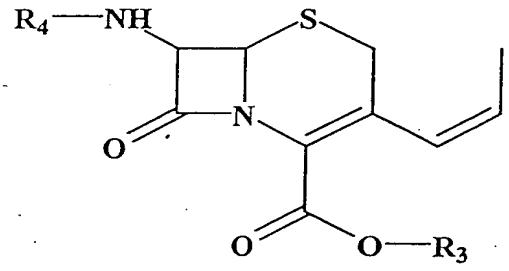
i) preparing the mixed acid anhydride of α-amino-p-hydroxy phenylacetic acid of formula (III) by a process comprising the steps of

(d) adding an acylating agent and a base to an inert organic solvent or a mixture thereof at a temperature in the range of 0° to 40°C;

(e) cooling the solution to a temperature in the range of –70° to –30°C;

(f) addition of Dane salt of α-amino-p-hydroxy phenylacetic acid to the cooled solution and agitation at a temperature in the range of –70° to –30°C;

ii) reacting the mixed acid anhydride of formula (III) thus prepared with a protected 7-APCA of formula (VII)



(VII)

wherein R_3 and R_4 are each trialkylsilyl group,

followed by hydrolysis, isolation and purification to give Cefprozil of formula (I) in the form of a monohydrate in high yield and purity, substantially free of impurities.

3. A process according to anyone of claims 1and 2, wherein the inert organic solvent or solvent mixture employed in step i)(a) is chosen from methylene chloride, tetrahydrofuran, chloroform, N,N-dimethyl formamide, diethyl ether, chlorotethane, acetonitrile, trichloroethylene, acetone, ethyl acetate.

4. A process according to anyone of claims 1and 2, wherein suitable acylating agents employed in step i)(a) is chosen from reactive forms of aliphatic, alicyclic, or aromatic acids such as chloroformic acid, benzoic acid, pivalic acid and 2-ethylhexanoic acid. The reactive forms of these acids include their esters such as ethyl chloroformate, isobutyl chloroformate and their halogenides like pivaloyl chloride, 2-ethyl-hexanoyl chloride and benzoyl chloride, the preferred acylating agent being ethyl chloroformate.

5. A process according to anyone of claims 1and 2, wherein the base employed in step i)(a) is selected from triethylamine, picoline, N-methylmorpholine, N,N-dimethylbenzylamine, lutidine, N,N-dimethyl-4-aminopyridine, N,N-dicyclohexylamine, the preferred base being N-methylmorpholine.

6. A process according to anyone of claims 1and 2, wherein the acylating agent employed in step i)(a) is employed preferably in the range of 1to1.5 moles per mole of Dane salt.

7. A process according to anyone of claims 1and 2, wherein the base employed in step i)(a) is employed preferably in the range of 0.02 to 0.04 moles per mole of Dane salt.

8. A process according to anyone of claims 1and 2 wherein the temperature in step i)(a) is preferably 20° to 25°C.

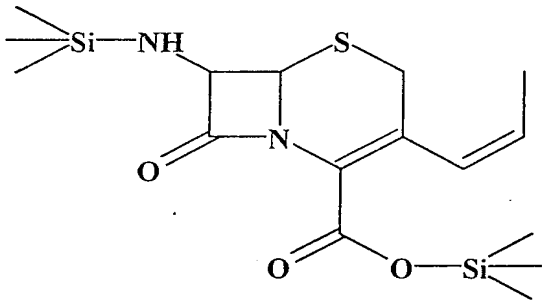
9. A process according to anyone of claims 1and 2 wherein the Dane salt is preferably sodium or potassium D-N- (1-methoxycarbonylpropene-2-yl)-α-amino-p-hydroxyphenyl acetate or sodium or potassium D-N-(1-ethoxycarbonylpropene-2-yl)-α-amino-p-hydroxyphenyl acetate.

10. A process according to anyone of claims 1 and 2 wherein the temperature in step i)(c) is preferably –35°C to –50°C.

11. A process according to anyone of claims 1and 2 wherein the mixed acid anhydride is condensed with protected 7-APCA at a temperature preferably in the range of –90° to –30°C.

12. A process according to anyone of claims 1, 2 and 11 wherein the mixed acid anhydride is condensed with protected 7-APCA at a temperature most preferably in the range –50° to –40°C.

13. A silylated 7-amino-3-(propen-1-yl)-3-cepham-4-carboxylic acid compound of formula (VI).



(VI)

Dated this 01st day of October 2003.

Meghna S. Vaidya
Of S. MAJUMDAR & CO.
Applicants' Agent